NHS Blood and Transplant

HISTOCOMPATIBILITY AND IMMUNOGENETICS DIAGNOSTIC SERVICES

USER GUIDE 2015

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User Guide for Histocompatibility and Immunogenetics Diagnostics Services

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CHAPTER 1: GENERAL INFORMATION

THIS GUIDE

The NHSBT H&I function offers an integrated package of services, testing and clinical advice across the whole H&I field from a network of six laboratories. Working as an integral part of NHSBT we offer hospitals an unrivalled portfolio of testing, advice and support in transplantation, supply of selected blood products and Immunogenetics.

This guide outlines the Histocompatibility & Immunogenetics (H&I) services provided by NHSBT and will be of use to consultants and other medical, nursing and scientific staff in transfusion laboratories, haematology departments, transplant units and other healthcare environments with patients requiring our services. The guide contains information about the organisation of services and contact details for key members of staff and other information to enable healthcare staff to access services on behalf of their patients.

QUALITY STATEMENT

The H&I function in common with other NHSBT services is committed to quality, as outlined in our Quality Policy document. All work is carried out within the framework of a documented quality system, according to good laboratory and good manufacturing practice (GLP, GMP), in compliance with the Blood Safety and Quality Regulations, Human Tissue (Quality and Safety for Human Application) Regulations (TQSR), EU Organ Donation Directive (EUODD), the Data Protection and Freedom of Information Acts. Techniques and procedures are validated, described in standard operating procedures (SOP), and conducted by staff whose proficiency is regularly monitored.

NHSBT Quality managers carry out regular audits to establish and improve the level of GLP and GMP compliance. These complement external licensing and accreditation inspections by the Medicines and Healthcare Products Regulatory Agency (MHRA), UKAS/Clinical Pathology Accreditation (CPA), European Federation of Immunogenetics (EFI) Human Tissue Authority (HTA), Care Quality Commission (CQC) and other relevant accreditation bodies.

The Head of Function, Heads of Laboratories, laboratory and support staff have continued to standardise practice and strive for a consistent and high quality service. Procedures are developed to work according to the principles of clinical governance.

All laboratories within the function participate in external quality assurance schemes such as UKNEQAS and where appropriate in international workshops. In some instances, this participation extends to the provision of source material, devising the exercises or acting as a reference laboratory.

COMPLIMENTS and COMPLAINTS

NHSBT is committed to continuously improving the quality and range of services provided and welcomes any comments or suggestions from the service users. There is always the risk of failures in any service delivery and it is essential that these be reported to ensure the causes can be fully investigated, to reduce the risk of recurrence, help improve the service and ensure compliance with clinical governance policies (specific forms have been made available to every service user for this and can be found on the NHSBT website at: http://hospital.blood.co.uk/customer-services/complaints-compliments-and-feedback/)

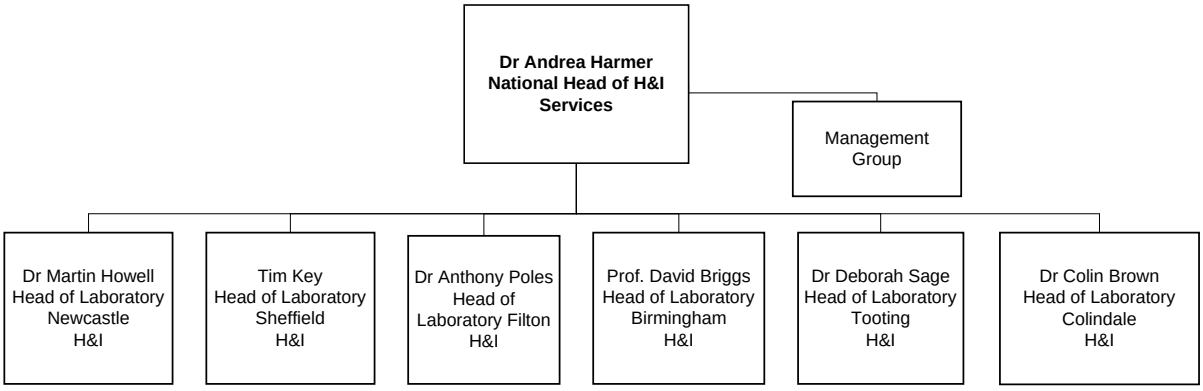
Please do not hesitate to discuss complaints with either your Customer Services Manager or the relevant Head of Laboratory. We always strive to provide a satisfactory response to any complaint. However, if you are unhappy with the handling of your complaint, then please contact the Head of Service Delivery or the Lead Quality Specialist for H&I.

Complaints must be clearly separated from communication about serious hazards of transfusion (SHOT) or near misses, which have or could have affected the quality of patient care. Such incidents and near misses often require immediate action and you are advised to discuss these with an NHSBT Medical Consultant or a senior laboratory scientist at your local blood centre. Serious events must be reported to the SABRE scheme.

CHAPTER 2: H&I LABORATORIES AND MANAGEMENT

There are six laboratories in the H&I Function with approximately 190 members of staff. The laboratories are located at NHSBT Birmingham, Filton (Bristol), Colindale (North London), Newcastle, Sheffield, and Tooting (South London) and each is directed by a Consultant Clinical Scientist. Figure 1 highlights the management structure of the H&I Function.

Figure 1: Diagram of the management structure of the H&I Function



NHSBT H&I laboratories support haematopoietic stem cell and solid organ transplant programmes at hospitals throughout England. The H&I laboratory at Filton provides platelet immunology and granulocyte immunology services nationally.

The H&I laboratory at Colindale works in co-operation with the British Bone Marrow Registry (BBMR) carrying out high throughput typing NHSBT's blood donors who have volunteered to become stem cell donors. The H&I laboratory at Colindale also performs the typing and registration of stem cell donors for the BBMR and cord blood donor units collected by the NHS-Cord Blood Bank (NHS-CBB). The HLA data is then submitted to Netcord and to Bone Marrow Donors Worldwide (BMDW).

All NHSBT H&I laboratories are accredited by Clinical Pathology Accreditation (UK) Ltd (CPA) and European Federation for Immunogenetics (EFI) for the clinical services they provide.

Table 1: License / accreditation numbers

	UKAS/CPA	EFI	MHRA (MIAIMP)*
Birmingham	West: 2821	03-GB-020.980	BE25224 site :21251
Bristol - Filton	West: 2821	03-GB-015.985	BE25224 site :698080
Colindale	South: 2822	03-GB-002.998	BE25224 site :90677
Tooting	South: 2822	03-GB-003.997	BE25224 site :90667
Newcastle	North: 2823	03-GB-004.996	BE25224 site :90668
Sheffield	North: 2823	03-GB-006.994	BE25224 site :21292

MIAIMP - Department of Health and MHRA Register of Holders of Manufacturer's Authorisations for Investigational Medicinal Products (MIAIMP) 2013

LABORATORIES 'OPENING HOURS'

The core working hours of the laboratories are between 9.00 a.m. and 5.00 p.m. from Monday to Friday, excluding bank holidays.

OUT OF HOURS

Solid Organ Transplant programmes
Out of hours on-call, is provided by H&I laboratories supporting solid organ transplant programmes, 24 hours a day, 365 days a year. A Consultant Clinical Scientist can be contacted on your request in case of clinical emergency by contacting your local NHSBT Hospital Services.

HLA Selected Platelets

Appropriate use of out-of-hours service

HLA selected platelets required for early delivery on weekdays and for transfusion at weekends should always be ordered within normal laboratory Monday-Friday working hours and with at least 24 hours notice. Should a clinical emergency (e.g. an episode of bleeding) occur 'out of hours' (i.e. evenings, overnight or weekends), an emergency 'on call' service for HLA selected platelet provision is available. However, since it is not possible to provide optimally HLA matched platelets at short notice, this service should be used for clinical emergencies only and not for ad hoc routine requests. If 'out of hours' provision is requested, it must be discussed and authorised by the NHSBT H&I Consultant on call, who will need to call in a staff member to process the request. Selection and provision of units is likely to take a minimum of 6 hours and may take longer. Whilst NHSBT will endeavour to provide a well matched unit finding a suitably matched unit at short notice may not always be possible for all patients.

Out of hours ordering of HLA Selected Platelets

If there is an urgent clinical need for out of hours transfusion in patients already receiving HLA selected platelets orders should be sent to Hospital Services.

Any orders for HLA selected platelets received by Hospital Services will be treated as **emergency** orders and will be passed on to an on call H&I Consultant to be dealt with as an emergency request.

The emergency service is available week-nights from 5.00 p.m. to 9:00 a.m. and weekends from 5:00 p.m. on Friday to 9:00 a.m. on Monday, and all day on bank holidays.

Please send **routine** orders directly to your local H&I laboratory to be processed during normal laboratory hours (Mon-Fri 9:00 a.m. – 5:00 p.m.).

The process for ordering HLA selected products is described on the NHSBT Hospital and Science website: http://hospital.blood.co.uk/library/request_forms/hla/order_hla/

CHAPTER 3: SAMPLE REQUIREMENTS & REPORTING

SAMPLE REQUIREMENTS

For sample requirements refer to Table 3.

REQUEST FORMS

There are five request forms available which are shown in Table 2.

Table 2: Request forms

		Request form
3A	H&I Diagnostic and transfusion laboratory	FRM745
3B	H&I Organ Transplant recipients and donors	FRM1008
3C	H&I Haematopoietic Stem Cell Transplantation	FRM1010
3D	H&I Platelet Immunology	FRM999
3E	H&I Granulocyte Immunology	FRM1001

Request forms can be ordered directly from your **local H&I laboratory** and are available to download from the NHSBT hospital website along with guidance documents on their correct completion: http://hospital.blood.co.uk/diagnostic-services/hi/hi-test-request-forms/ . A process of review and improvement of these forms is currently underway with new versions due out early in 2015. Requesters are advised to withdraw the previous versions of updated request forms when they are released as the use of out of date paperwork may cause errors in sample distribution.

SAMPLE COLLECTION AND LABELLING

No specific clinical patient preparation is needed for sample collection for H&I testing. All materials used in sample collection should be disposed of safely following local sharps and clinical waste procedures.

A request form must accompany every sample. Samples with different collection dates, of different sample types (e.g. bone marrow aspirate or peripheral blood) or from different individuals (including family members) must each be accompanied by their own test request forms. Request forms are the basis to establish the correct identification of the patient. Schemes, such as the SHOT scheme, have shown that serious incidents are often caused by errors of a clerical nature.

The points of identification provided on the request form must match the information provided on the sample. Due to the increased risk of mislabelling when using pre-printed labels (addressographs), samples for platelet transfusion investigations can not be accepted when the sample is labelled with an addressograph, for our full sample labelling policy see MPD1108.

MPD1108 section 1.4

“Only labels that are printed ‘on demand’ and attached to the sample tube next to the patient at the time of phlebotomy are acceptable. Since it is not possible to distinguish reliably between these and *addressograph* labels they can be accepted only from referring organisations which have informed NHSBT, in writing, that their sample labels are generated in an audited system and are demand printed at the time of phlebotomy. Bedside generated labels need to have positive, traceable identification of the sample taker, but do not require a signature.”

The laboratories may not accept referrals with inadequately completed request forms or incomplete sample labelling or where sample and request details do not match.

In case of a clinical emergency, NHSBT may agree with the requesting consultant or laboratory scientist to proceed with the requested investigations. However, in such cases the issue of blood products and laboratory reports will carry an explicit warning that the three points of identification were not used for the samples and/or request form and responsibility for correct identification of the sample

and patient lies with the requester such sample testing may be delayed while the laboratory confirms the sample details. The requester is advised to check the identifiers and to obtain reassurance about the identifiers used for the linking between patient and sample.

The following information is mandatory* on samples:

- Surname and forename in full
- Date of birth
- NHS number
- Date, and time if pertinent, of sample collection

*Certain exceptions apply, e.g. anonomised samples with a unique identifier such as a GUM clinic patient test request or for non UK nationals where no NHS number exists, for full details see our management process description document MPD1108 available on our website (http://hospital.blood.co.uk/diagnostic-services/hi/)

From April 2013, the Department of Health has stipulated that NHS organisations are expected to use the NHS number consistently and therefore NHS number is requested to be provided on all samples and request forms.

The following additional information is also required on the request form:

- Requesting hospital name in full (including town or city)
- Known risk sample
- NHS or Non-NHS
- Type of investigations requested
- Diagnosis/treatment
- Type of sample if not peripheral blood

NHSBT should be informed if samples are from non-NHS patients. The terms and conditions of service provision for the NHS by NHSBT are agreed with the National Commissioning Group. Service provision for non-NHS patients may be charged differently.

Clinical information is essential for providing the most appropriate testing and advice. The quality of clinical advice will also depend on provision of adequate clinical information. Absence of clinical information may lead to a delay in the processing of the sample while the requester is contacted to clarify or ascertain the type of investigations required.

NHSBT stores patient data on a national database and the use of hospital number without other points of identification may lead to errors as a hospital number is not unique. NHS number must be used in accordance with Department of Health requirements except in cases where the individual has no NHS number or where anonymity is mandated. If the address is provided then it may be entered on the patient's NHSBT computer record and appear on some patient reports. If the address contributes to the quality of identification then it may be used as a form of identification.

The sample needs to be dated as this information can be significant in determining the advice we will issue, in addition the outcome of some tests may be influenced by the age of the sample.

KEY FACTORS THAT MAY AFFECT TESTING

Sample storage time: In general, samples should be sent to the laboratory with minimum delay and to arrive within 24 hours of sample collection.

Sample storage and transportation temperature: In general, samples should be stored and transported at ambient temperature.

It is important to collect samples into the correct tubes. Please ensure the correct anticoagulant (usually EDTA) or no anticoagulant (clot) is used. It is also important to supply adequate volumes of blood to allow completion of testing (sample types and volumes are listed in Table 3).

In addition, if the patient's platelet and/or white cell count is low, or affected by condition and/or drug regime, this may affect the outcome of some tests.

For further details please refer to NHSBT H&I test request forms and information in Table 3: Summary of volumes and type of blood samples required for each test or contact your local H&I laboratory for help and advice.

Volume and types of sample

Refer to Table 3 for the volumes and type of blood samples required for each test. For further information look at the request forms via the NHSBT hospital website. Contact the relevant laboratory when referring samples of infants under 6 months to discuss the minimum sample requirements.

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Table 3: Summary of volumes and type of blood samples required for each test

TEST	SAMPLE REQUIREMENTS	LABORATORY
Transfusion & transfusion reactions		
*Initial investigation of Platelet refractoriness	6ml EDTA and 6ml clot	Local H&I laboratory
* NB this investigation requires an HLA type and HLA antibody screen		
Follow up testing of platelet refractoriness	6ml clot	
HLA type	6ml EDTA	
HLA antibody screen	6ml clot	
Transfusion-Related Acute Lung Injury (TRALI)	Pre-transfusion sample 2 x 6ml clot & 2 x 6ml EDTA (patient) Donation numbers of all blood products transfused < 24hrs before event	Filton
Transfusion-Associated Graft Versus Host Disease (TA-GVHD)	Discuss sample requirements with H & I Consultant	Local H&I laboratory
Post transfusion purpura (PTP)	6ml EDTA and 6ml clot	Filton
Granulocyte immunology		
Neonatal AlloImmune Neutropenia (NAIN) for initial screen – not crossmatch	Maternal = 6ml EDTA + 6ml clot Paternal = 6ml EDTA Neonate = 0.5-1ml ETDA	Filton
Autoimmune neutropenia	6ml clot (smaller volumes permissible for infants)	
Drug related neutropenia	6ml clot + sample of drug(s) Contact the laboratory before referring samples	
Platelet immunology		
Foetal/Neonatal AlloImmune Thrombocytopenia (NAIT) for initial screen – not crossmatch	Maternal = 6ml EDTA + 6ml clot Paternal = 6ml EDTA Neonate = 0.5-1ml ETDA	Filton
Heparin Induced Thrombocytopenia (HIT)	6ml clot	
Other drug related thrombocytopenias	6ml EDTA and 6ml clot + sample of drug(s) Contact the laboratory before referring samples	
Autoimmune thrombocytopenia	3 x 6ml EDTA and 6ml clot contact laboratory	
Thrombasthenias	Contact the laboratory before referring samples	
Haematopoietic stem cell transplantation		
Patients HLA type	6 – 80 ml EDTA * *Depending on WBC count.	Local H&I laboratory
Donor HLA type	6ml EDTA	
HLA- specific antibodies	6ml clot	
Chimerism analysis	6ml EDTA	
Solid organ transplantation		
HLA type of patients, donors or family members	6ml EDTA	Local H&I laboratory
HLA-specific antibodies	6ml clot	
Cross-match - live donor	40ml EDTA (donor)	
	6ml clot (recipient)	
Cross-match - deceased donor	6ml clot (recipient)	
	60ml EDTA (donor) OR spleen or lymph node as appropriate	
Newcastle require Li^+ Heparin INSTEAD of EDTA for cross matching		
Auto cross match	20ml EDTA + 6ml clot (recipient)	
ABO group	6ml EDTA	
ABO grouping can be sent directly to NHSBT RCI laboratories or H&I laboratories can forward them to RCI on your behalf. A charge will be levied for grouping. NHSBT RCI User Guide can be accessed at http://hospital.blood.co.uk/library/user_guides/index.asp		
Immunogenetics		
HLA typing for disease association and drug hypersensitivity	6ml EDTA	Local H&I laboratory

TYPE OF REQUEST

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The type of request and reason for the request must be clearly identified on the appropriate request form.

CONSENT

To comply with the Human Tissue Act legislation (Human Tissue Act, 2004), it is the responsibility of the requester to ensure that any patient or donor has been informed of, and has consented to, the tests being requested.

NHSBT may ask the requester to provide a copy of this information. Patients/donors should be informed that any residual material of a sample may be stored as part of required archiving protocols or to enable further investigation for the benefit of the individual. They also must be informed that excess surplus material may be used anonymously for quality control purposes, service development or education, and / or ethics committee approved research projects.

Where patient or donor consent is required it is the responsibility of the requester to ensure the subjects of any tests have given informed consent. Unless written notice is received to the contrary, consent for investigations and the use of any surplus sample in scheduled purposes (quality control, staff development or ethics committee approved research) will be assumed.

NHSBT H&I laboratories have developed a series of patient information leaflets to assist healthcare professionals to obtain informed consent for diagnostic testing. The leaflets explain what happens to their samples and why the tests are undertaken. In addition, there is a brief explanation of Histocompatibility & Immunogenetics investigations. The leaflets are available to download from the NHSBT hospital website and / or hard copies can be ordered directly from your local H&I laboratory. The link for patient information leaflets is: http://hospital.blood.co.uk/diagnostic-services/hi/patient-information-leaflets/

Table 4: Summary of H&I patient leaflets

Patient Information leaflet		Laboratory
Histocompatibility testing for kidney transplant donors	INF253	Local solid organ H&I laboratory
Histocompatibility testing for kidney transplant patients	INF255	
Histocompatibility testing for cardiothoracic transplant patients	INF254	
Histocompatibility testing for platelet transfusion patients	INF256	Local H&I laboratory
Histocompatibility testing for stem cell transplant patients	INF257	
Histocompatibility testing for possible donors or relatives of stem cell transplant patients	INF258	
Immunogenetic markers and diagnosing diseases	INF259	
Heparin-induced thrombocytopenia (HIT) : Your background guide to HIT and the associated laboratory testing	INF260	Filton
Information for mothers about neutrophil blood groups and Neonatal alloimmune Neutropenia (NAIN)	INF261	
Platelet groups & antibodies in pregnancy	INF283	

PACKAGING AND TRANSPORT

It is the responsibility of the sender to ensure that all samples are packaged in accordance with the current European agreement concerning Carriage of Dangerous Goods by Road Regulations, packaging instructions 650, to prevent breakage or spillage in transit. The outside of the box or package containing the samples must be clearly addressed to the H&I Department at the appropriate Blood Centre where the testing laboratory is based including “H&I Department - Diagnostic Samples” as the first line of the address to prevent delivery to H&I sample reception being delayed. This may not be your local blood centre. NHSBT reserves the right to refuse to handle any samples which are inappropriately packaged or labelled; customers sending unsatisfactorily packaged samples will be contacted.

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Pre-printed address labels can be provided on request from Customer Services. For advice from the Health and Safety Executive (HSE) on packaging for posting samples see: http://www.hse.gov.uk/biosafety/blood-borne-viruses/transportation-of-infectious-substances.htm

WHERE TO SEND SAMPLES

Routine samples
Accurate completion of the request form and clear labelling is essential for an effective transfer of samples to the testing laboratory. Samples for non-urgent testing can be given to NHSBT blood delivery driver via the hospital transfusion laboratory. For investigations not available from your local blood centre it is advisable to send samples directly to the H&I laboratory conducting the testing. Samples for platelet immunology and granulocyte immunology investigations should be sent directly by first class post to the H&I laboratory at Filton. Refer to Table 2 for further details.

Urgent samples
For urgent testing of samples **please phone the testing laboratory** and discuss the arrangements for sending the samples. Urgent samples should be transported directly from the hospital transfusion laboratory, transplant unit or requesting clinician to the blood centre where tests are performed. Packages must be clearly labelled (including "H&I Department - Diagnostic Samples") to ensure samples do not go astray. Blood Centre location maps can be provided on request or from the NHSBT website for couriers carrying urgent samples.

REPORTING TIME

In 90% of cases NHSBT aim to issue reports for investigations such as general H&I, platelet immunology, DNA based investigations e.g. HLA, HFE and other immune polymorphism typing within five working days **from receipt of the samples in the laboratory**. A longer turnaround time may apply to other investigations.

HLA specific antibody test reports for patients refractory to platelet transfusion will normally be issued within seven working days, but preliminary reports of HLA antibody positivity may be available sooner upon discussion with the local laboratory.

Drug dependent antibody screening (other than heparin induced thrombocytopenia) may take up to 20 working days, as these investigations often require additional studies.

Reports for complex cases e.g. requiring multi-stage testing, family or combined donor/recipient reports requiring collation of test results from multiple samples may take longer than five days from receipt of the first test request/sample to the generation of the final report.

In 90% of cases NHSBT aim to issue reports for granulocyte immunology investigations within 14 working days **from receipt of the samples in the laboratory**. If further (specific) investigations are required, the turnaround time may extend to 21 working days.

Blood samples referred for foetal HPA typing will be tested and the results reported within 3 working days of receipt of the sample. Amniocytes referred for foetal HPA typing will be tested and the provisional results reported within 3 working days of the receipt of the sample. A final report is only issued after typing of cultured amniocytes. Depending on the number of viable cells, a further 21 days may be required before sufficient cells are available for confirmatory typing to be completed.

Details are summarised in Table 5.

Table 5: Summary of reporting time

SERVICE	REPORT	90% within
Immunological refractoriness to platelets	HLA type	5 working days
	HLA antibody screen	7 working days
Platelet Immunology (PI)	Platelet antibody specificity (e.g. NAIT) **	5 working days
	HIT	5 working days
	HIT - urgent result *	1 working day
	Other drug induced thrombocytopenia	20 working days
	Foetal HPA typing	3 working days
Granulocyte Immunology (GI)	All tests	21 working days
Haematopoietic Stem Cell Transplantation (HSCT)	HLA type - class I and II	7 working days
Solid organ transplantation	HLA type - class I and II	7 working days
	HLA antibody screen	15 working days
	Urgent result	1 day
Immunogenetics	All tests	5 working days

* This must be discussed with the laboratory ahead of sending the sample
** HPA -15 antibody testing may require 21 working days depending on donor availability

COMPUTER RECORDS AND REPORTS

Computer records

The H&I laboratories are supported by national computer systems (Hematos/PULSE) on which patient and donor data are stored. NHSBT computer systems are registered under the Data Protection Act. Access to the database is on a 'need to know' basis for 'clinical care purpose only' and confidentiality is respected at all times.

Reporting

NHSBT H&I now has electronic reporting capabilities for those requesters with N3 NHS network access. This system is based on the Sunquest ICE electronic reporting system and is named SpICE (Specialist Services ICE).

SpICE will reduce significantly the time taken for reports to be available to requesters once they have been authorised for release, all reports should be visible to those with access to them on the system within one hour of the report being authorised as opposed to being delayed by the printing and postal delivery process. Hard copy reports will continue to be sent, in addition to the electronic reports, until NHSBT is informed by requesters that they are no longer required.

The basic principle for hard copy reports is for them to be sent to the requester. Please contact the laboratory if you require different hard copy reporting arrangements. When requested, urgent reports can be faxed to a requester but the requester will be asked to fax this request on headed paper as proof of identity in order to protect patient confidentiality.

More information about SpICE, including a user guide, presentation and FAQ can be found on the NHSBT Hospital and Science website at: http://hospital.blood.co.uk/diagnostic-services/sp-ice-browser/

Antibody cards

For patients with clinically relevant platelet (HPA) and / or neutrophil (HNA) allo antibodies or cell specific iso or drug dependent antibodies, an antibody card will be issued for the patient. SpICE will enable hospitals to access patient antibody cards for printing if required. Information leaflets will be sent for patients with a diagnosis of NAIT, NAIN or HIT. Antibody cards are not issued for patients with HLA specific antibodies.

CHAPTER 4: H&I SERVICES

Services are provided to support the diagnosis and/or treatment of a variety of conditions and are relevant in the following areas of clinical medicine shown in Table 6.

Table 6: Areas of clinical medicine involving H&I services

	CHAPTER
H&I service relating to transfusion and transfusion reactions Platelet refractoriness and provision of HLA/HPA selected platelets Investigations of serious hazards of transfusion (SHOT)	4A
Platelet Immunology (PI)	4B
Granulocyte Immunology (GI)	4C
Haematopoietic Stem Cell Transplantation (HSCT)	4D
Solid organ transplantation	4E
Immunogenetics	4F

CHAPTER 4A: H&I SERVICES RELATING TO TRANSFUSION & TRANSFUSION REACTIONS

PLATELET REFRACTORINESS AND THE PROVISION OF HLA/HPA SELECTED PLATELETS

Platelet transfusion refractoriness may result from immune or non-immune platelet destruction. The identification of platelet refractoriness due to HLA/HPA specific antibodies is important to enable allocation of these specialised products to those patients who will benefit from them. Patients with non-immune platelet refractoriness will not gain any additional benefit from HLA/HPA selected platelets compared to non-HLA selected platelet units. In some patients with HLA specific antibodies, HPA specific antibodies may also be present requiring donor platelets compatible with both types of antibodies. If compatible platelets cannot be provided, either increasing the transfused dose or discontinuing prophylactic platelet support may be appropriate strategies.

CMV negative selected products
The Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO) position statement on the provision of cytomegalovirus tested blood components recommends that for patients other than neonates (under 28 days and interuterine transfusions) leucodepleated products provide adequate risk reduction for the transmission of CMV.

Quote from SaBTO position statement:

"All blood components (other than granulocytes) in the UK now undergo leucodepletion, which provides a significant degree of CMV risk reduction. This measure is considered adequate risk reduction for all other patients requiring transfusion (haemopoietic stem cell transplant patients, organ transplant patients, and immune deficient patients, including those with HIV) without the requirement for CMV seronegative components in addition."

The full report of the SaBTO CMV Steering Group may be found at:
https://www.gov.uk/government/publications/sabto-report-of-the-cytomegalovirus-steering-group

Restricting selected products to CMV seronegative units severely reduces the number of units available and may result in a less beneficial unit being selected for your patient.

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Alloimmunisation against platelets
Alloimmunisation is defined as the development of an immune response against alloantigens. In some transfusions this immune response may result in the production of HLA and/or HPA specific antibodies. Refractoriness is the failure to obtain satisfactory responses to transfusions of platelets from unselected but ABO compatible donors. A proportion of, but not all, alloimmunised patients will become refractory. It is generally accepted that as a consequence of universal leucocyte depletion of all blood components the rate of alloimmunisation has dropped to approximately 10-25%. However the precise incidence is influenced by a number of factors including pregnancies and the number of transfusions. Non-immune mechanisms are an important cause of refractoriness and have been shown to cause transfusion failure in a significant group of patients on prophylactic platelet transfusion support.

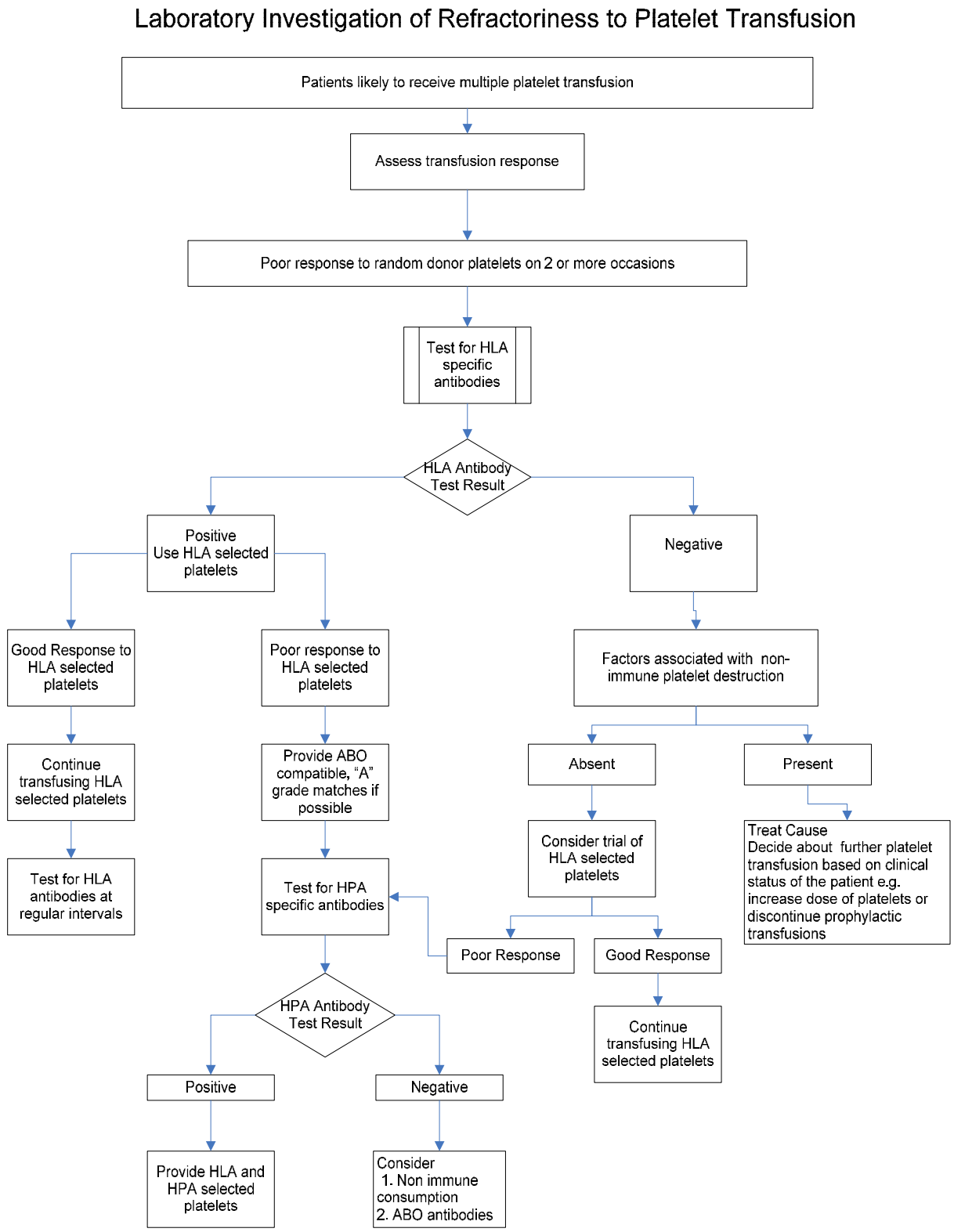
Platelet refractoriness
Platelet refractoriness is defined as a failure of the platelet count to increase by greater than 10 x 10^9/L at between 1 and 24 hours after the transfusion of an adult dose of ABO compatible platelets (> 240 x 10^9 /L platelets). Refractoriness to random donor platelets can be of non-immune or immune cause, or a combination of both.

When to request HLA class I selected platelets
The majority of patients with immune refractoriness are best supported with platelet transfusions that are either HLA selected or HLA compatible between the donor and patient. HLA selected platelets are collected by apheresis and specific donors may have to be called to donate these platelets for a specific patient. The provision of this service is time consuming and expensive and should be reserved for those patients who really need them.

The following criteria should be met:

- Exclusion of non-immune causes of refractoriness
- Positive screen for HLA class I or HPA specific antibodies or both
- Refractoriness to an ABO compatible platelet concentrate on two occasions

Figure 2: Laboratory Investigation of Refractoriness to Platelet Transfusion



Increments with HLA class I selected platelets

Transfusion of selected platelets in patients with immune refractoriness results in a significantly improved post-transfusion increment in 60-70% of patients. The H&I laboratory needs to collect increment data to identify units that obtain satisfactory results from transfusions. The platelet count should be measured 1 hour after completion of the transfusion but can be obtained after 10 minutes (1). The laboratory can then identify those donors that are most beneficial to patients and assign further units accordingly. Transfusion failure with HLA class I selected platelets may be due to co-existing non-immune causes of refractoriness, HPA alloantibodies, platelet autoantibodies, drug-dependent platelet antibodies and potent anti-A or anti-B antibodies. If increments with HLA class I selected platelets are poor, the case should be discussed with an H&I Consultant Clinical Scientist. Assays for the detection and identification of HPA specific antibodies may then be recommended. In refractory patients with active bleeding, a dual investigation strategy of simultaneous investigations for HLA and HPA specific antibodies may be indicated.

HLA & HPA Selected Platelets

For patients with HPA as well as HLA class I specific antibodies, attempts will be made to provide dual selected platelets. This may not be possible if the platelet specific antibodies are against high frequency HPA alloantigens. In this case, HPA selected platelets alone may be provided to determine if these give a satisfactory increment.

Ordering HLA & HPA Selected Platelets

Orders for selected platelets should be made during normal working hours and wherever possible at least **24 hours notice** should be given. Planning in advance allows the H&I laboratory to source the best available HLA/HPA selected product for the patient. The units most appropriate for selection may not be in the stock held at the local NHSBT centre and may require transport from another NHSBT centre to your local centre for issue. Products which have to be supplied at short notice may not always be the optimal product for the patient. Outside normal working hours platelets can be supplied for existing patients in cases of clinical urgency only.

SERIOUS HAZARDS OF TRANSFUSION (SHOT)

The overall incidence of serious side effects is small when compared with number of blood components used per annum by the NHS. In the majority of cases of serious reactions or hazards associated with transfusion the diagnosis is a clinical one and in some elaborate laboratory tests are required to confirm the diagnosis. A suspected adverse reaction should be discussed in the first instance with a NHSBT Consultant Haematologist to agree on the best set of laboratory investigations. Samples can be referred to one NHSBT laboratory and will be distributed internally.

Reporting adverse reactions to transfusions

There is a regulatory requirement in the UK under the terms of the Blood Safety and Quality Regulations 2005 to report adverse reactions related to transfusion. The Medicines and Healthcare products Regulatory Agency (MHRA) has been appointed the Competent Authority on behalf of the Secretary of State to administer the regulations, and has developed a web-based haemovigilance reporting system called SABRE (Serious Adverse Blood Reactions and Events) to facilitate reporting.

All Trusts in the UK should be registered with the MHRA and must submit a 'notification' report to them as soon as possible following a reaction. At the time of reporting, there is the opportunity to tick a box which allows the report to be transmitted to SHOT (the Serious Hazards of Transfusion confidential enquiry) allowing them access to the report details. All trusts should be registered to report to SHOT's web based database, known as Dendrite. Reporting to SHOT is strongly encouraged and is professionally mandated by accreditation bodies such as CPA (Clinical pathology Accreditation).

[1] O'Connell B, Lee EJ, and Schiffer CA, The value of 10 minute post transfusion counts. Transfusion 1988;28:66-67

After selecting the SHOT option in SABRE, an automated e-mail is generated containing a link to the SHOT database (Dendrite). This SHOT questionnaire can then be completed online. Automated reminders will be sent at regular intervals until the SHOT questionnaire is completed.

Following investigation of the incident by the reporting hospital, and where appropriate by the blood services, the reporter is required to submit a 'confirmation' report to MHRA via SABRE which effectively closes the case, provides an assessment of the likelihood of the reaction being due to the blood component and details, where appropriate, any corrective and preventative actions put in place to reduce the likelihood of the event recurring.

Further information:
SHOT – www.shotuk.org, 0161 423 4208
MHRA – www.mhra.gov.uk, 020 3080 6000

Current SHOT reporting categories and laboratory flowchart may be found at:
http://www.shotuk.org/sabre/
The SHOT reactions that involve the H&I laboratories in the investigations are in Table 7.

Table 7: SHOT Categories that involve the H&I laboratories in the investigations

Type of SHOT reaction	Abbreviation
Post transfusion purpura	PTP
Transfusion-associated graft versus host disease	TA–GVHD
Transfusion related acute lung injury	TRALI
Severe non-haemolytic febrile transfusion reactions (NHFTR) Classified as Acute transfusion Reaction by SHOT	ATR

TRANSFUSION ASSOCIATED GRAFT VERSUS HOST DISEASE (TA-GVHD)

Transfusion Associated Graft Versus Host Disease (TA-GVHD) is usually fatal but almost entirely preventable complication of transfusion. Patients at risk of this complication have been clearly defined, as have groups not considered to be at risk. Components implicated are red cells, platelet concentrates, fresh plasma and granulocytes. At risk patients should carry the card issued by the Department of Health, which can be obtained from NHSBT Hospital Services, and receive gamma-irradiated blood components. The dose of gamma irradiation should be a minimum of 2500 cGy to any part of the blood component.

Investigations
In supporting the clinical diagnosis, laboratory testing to demonstrate mixed chimerism is important. TA-GVHD is the result of engraftment and proliferation of alloreactive donor lymphocytes in the recipient. Inflammation and tissue damage follow. Tests for short tandem repeats on patient DNA and on DNA from pinch skin biopsy samples from affected and non-affected sites will be required to establish the presence of infiltrating donor lymphocytes in the TA-GvHD skin lesions and to unequivocally identify cells of donor and patient origin.

TRANSFUSION RELATED ACUTE LUNG INJURY (TRALI)

Transfusion Related Acute Lung Injury (TRALI) is a serious complication of transfusion which usually occurs within 6 hours of a transfusion episode and is characterised by symptoms and signs of dyspnoea, cyanosis, hypoxaemia and pulmonary oedema and in the absence of other causes such as cardiac insufficiency and fluid overload. Chest X-ray shows characteristic pulmonary infiltrates. None of the clinical features are specific to TRALI and the diagnosis is essentially clinical. The clinical presentation is indistinguishable from the Acute Respiratory Distress Syndrome (ARDS) or its less severe form, acute lung injury (ALI). The aetiology of TRALI is complex and is difficult to distinguish from ARDS/ALI on the basis of clinical symptoms and tests. TRALI is therefore a diagnosis made by

exclusion where other causes of ARDS/ALI are not apparent and where there has been a recent transfusion of blood or other plasma containing blood products. Although rare, TRALI is a significant cause of transfusion associated morbidity and mortality. The risk of the latter can be reduced by early recognition of the cause and optimal treatment. Some cases were considered, after review, not to be TRALI, illustrating the difficulty of making a positive clinical diagnosis of the condition. However in many cases, TRALI was thought either likely or possibly to have contributed to the patient's death. Leucocyte antibodies in the donor plasma generally cause this syndrome. Even a small volume of plasma containing leucocyte antibodies such as that found in SAG-M red cell concentrates is able to precipitate a reaction. On rare occasions TRALI can also be caused by leucocyte antibodies in the recipient or by immune complexes of leucocyte antigens and antibodies in platelet concentrates derived from pooled buffy-coats.

Investigations
The logistics of TRALI investigations are complicated and time consuming. When referring a suspected case of TRALI full clinical details should be provided in order to assess the likelihood of the reaction having been due to TRALI. Clinical details should include, nature of transfusion reaction and time in relation to transfusion, components transfused including donation numbers, treatment given including ventilation and clinical response.

Leucocyte antibodies are generally against HLA class I antigens but HLA class II or HNA specific antibodies may also be implicated. Initial investigations will be performed with fresh donor samples. It is therefore important that donation numbers of all implicated units (blood, platelet concentrates, FFP) in the 24 hours preceding TRALI presentation are provided. Pre- and post-transfusion serum samples from the patient should be provided, together with the date and time the samples were taken.

The investigations for TRALI are done at the H&I laboratory at NHSBT Filton. The investigations aim to identify the presence of:

- HLA class I and class II specific cytotoxic antibodies
- HLA class I and class II specific non-cytotoxic antibodies
- Granulocyte-specific antibodies

If donor leucocyte alloantibodies are detected then appropriate tests for the presence/absence of the antigen or allele in the patient/donor will be performed to determine whether the patient is positive for the cognate antigen. Even if this is the case, there is a good chance that the incompatibility is by chance and is not the cause of ARDS/ALI. TRALI does not always ensue even when a patient is positive for the cognate antigen.

Future transfusions
There is no clear evidence on the best transfusion support policy for patients who have experienced TRALI. However, the notion that in addition to donor leucocyte antibodies, patient factors may contribute to the risk of TRALI is generally accepted. Therefore, in a patient who has experienced a TRALI, it is recommended not to use plasma containing blood products from female donors (FFP, cryoprecipitate, platelet concentrates) as the chance of leucocyte antibodies being present is greater in this group.

POST-TRANSFUSION PURPURA (PTP)

Post-transfusion purpura (PTP) is a rare but serious transfusion reaction occurring 5 to 12 days after the transfusion of blood. A sharp decline in the number of confirmed PTP cases has been observed since the introduction of universal leucocyte depletion. PTP mainly occurs in women and HPA-1a specific antibodies are generally detected.

However, other HPA specific antibodies can also cause PTP. Severe thrombocytopenia occurring immediately after the transfusion of whole blood, a platelet concentrate or fresh frozen plasma can be caused by potent HPA antibody in the transfused plasma. All cases in which there is a precipitous fall

in the platelet count either immediately or some days after transfusion (except in case of massive transfusion) should be referred for investigations and reported. Patients who require blood peri-operatively and in whom a severe thrombocytopenia develops will often also receive heparin. However, the development of thrombocytopenia in PTP is more precipitous than in HIT (Heparin induced thrombocytopenia) and purpura and bleeding are characteristic of PTP. If PTP investigations are requested then it is important to inform the laboratory whether the patient was receiving heparin, even if this was only to flush an in-dwelling line.

Investigations
Tests for HPA specific antibodies and where appropriate, heparin-platelet factor 4 specific antibodies will be performed.

Therapy
High dose intravenous immunoglobulin (1.0 g/kg body weight on two to three consecutive days) is the treatment of choice. Platelet transfusion is usually contra-indicated in the acute phase. Plasmapheresis needs to be considered as an additional therapy if intravenous IgG does not result in a satisfactory rise of the platelet count. High dose corticosteroids are not recommended.

Transfusion support
In the acute phase of PTP, random ABO/D compatible blood components are advised. HPA compatible blood and platelets must be used if a patient requires transfusion after recovery.

SEVERE NON-HAEMOLYTIC FEBRILE TRANSFUSION REACTIONS

The incidence of Non-Haemolytic Febrile Transfusions Reactions (NHFTR) and of rigors have both reduced as a consequence of the introduction of universal leucocyte depletion. However, it remains a common consequence of transfusing blood or blood products. In the majority of cases pre-medication with paracetamol may alleviate symptoms. If severe and when combined with other features such as hypotension then bacterial contamination of blood products (especially platelet concentrates) must be considered and an NHSBT Medical Consultant must be contacted urgently for advice and investigations.

Non haemolytic febrile and allergic transfusion reactions with an immunological cause
Apart from bacterial contamination, severe febrile transfusion reactions may be caused by immunological reactions. Severe immunological reactions can be of the allergic / anaphylactic type with rashes, wheezing or dyspnoea. If febrile reactions recur and are refractory to paracetamol and corticosteroids other causes should be considered. It is advised that such cases be discussed with a NHSBT Medical Consultant. The straightforward method for prevention of both types of reactions is to alter the specification of the blood product; i.e. reactions to platelet transfusions may be simply resolved by replacement of the plasma by platelet suspension medium and to blood transfusions by removing the plasma proteins by washing.

If severe febrile reactions are not resolved by altering of the component specification then tests for leucocyte and platelet alloantibodies may be of use. If any of these antibodies are present in the patient then reactions may be remedied by better matching. In such rare and complex cases it is recommended to run investigations for HLA class I and class II, HNA and HPA specific antibodies in parallel. Samples should be referred to the local H&I laboratory. Tests for leucocyte and HPA alloantibodies have a low diagnostic specificity for NHFTR and the reactions may persist even if better selected blood or platelets are provided.

CHAPTER 4B: PLATELET IMMUNOLOGY (PI)

There are six clinical syndromes for which services are provided, as shown in Table 8.

Table 8: Clinical syndromes involving platelet immunology services

Neonatal alloimmune thrombocytopenia
Post transfusion purpura* (refer to chapter 4a)
Refractoriness - HPA only tested for after HLA antibody investigation * (refer to chapter 4a)
Delayed engraftment of platelet lineage following bone marrow transplantation – investigated only after HLA antibody investigation
Autoimmune thrombocytopenia – selected cases only
Drug-induced antibody mediated thrombocytopenia e.g. heparin, antibiotics, quinine and gold
Congenital and acquired thrombasthenias

Neonatal alloimmune thrombocytopenia (NAIT)
The frequency of Neonatal Alloimmune Thrombocytopenia (NAIT) is 1 in 1100 live births and is the most likely cause of severe thrombocytopenia in a term and otherwise healthy neonate. NAIT is caused by maternal IgG alloantibodies directed against a HPA antigen present in the foetus/neonate and absent in the mother. Many alloantigen systems have been described but the HPA-1a antigen is clinically most important and approximately 80% of severe cases are caused by anti-HPA-1a. Approximately a further 15% of NAIT cases are due to HPA-5b alloimmunisation and the strategy of providing HPA-1a (-), 5b(-) platelets in suspected NAIT cases will therefore be successful in 95% of cases involving Caucasians.

NAIT due to antibodies against HPA other than HPA –1a and – 5b and to isoantigens in the case of maternal platelet glycoprotein deficiencies account for approximately 5% of cases. In cases where antibodies to the major HPA are not detected and there is strong clinical evidence to support a diagnosis of NAIT, the maternal serum is also investigated for the presence of antibodies against 'private' or low frequency antigens by performing a crossmatch between maternal serum and paternal platelets.

Investigations for NAIT
The maternal serum will be screened for HPA specific antibodies using both an immunofluorescence test and a glycoprotein specific ELISA (MAIPA assay) with a panel of HPA and HLA typed platelets and when appropriate paternal platelets as soon as samples arrive at the laboratory. If the mother is found to have HPA specific antibodies, these results will be relayed to the requester as soon as possible. However, laboratory results should not delay transfusion of HPA-1a (-), 5b(-) platelets if NAIT is suspected and there is evidence of bleeding or if the platelet count is $<30 \times 10^9$/L. Maternal and paternal blood (and from infant if available) will be genotyped for the HPA -1, 2, 3, 4, 5, 6, 9 and 15 alleles.

Therapy
In a term neonate with normal clotting but severe thrombocytopenia ($< 30 \times 10^9$/L) or clinical signs of bleeding, the count should be corrected as soon as possible by transfusion of HPA-1a and HPA-5b negative donor platelets, without waiting for the results of the laboratory investigations. HPA-1a and HPA-5b negative platelets suitable for neonatal use are available 'from the shelf'. These platelets will be compatible with maternal HPA specific antibodies in over 90% of NAIT cases. If HPA-1a and HPA-5b negative platelets are not available from stock then normal ABO and D compatible donor platelets should be administered together with high dose intravenous immunoglobulin.

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Counselling and clinical questionnaires
If HPA alloantibodies are detected in the maternal serum, counselling should be provided to the parents about the risks to further pregnancies. Details of clinical outcome are sought by the laboratory in each confirmed case of NAIT.

Foetal HPA genotyping in future pregnancies if the partner is heterozygous
The HPA status of a foetus can be identified by analysis of genomic DNA derived from foetal blood or, amniotic fluid. Please discuss with one of the Consultant Haematologists before a decision for sampling is taken. In general, a 10ml sample of amniotic fluid (depending upon gestational age) or a chorionic villus biopsy is required. This should reach NHSBT H&I Filton within 48 hours of sampling. To avoid the possibility of contamination, it is preferable to dispatch the amniotic fluid without transferring it to a second container. If amniotic fluid is transferred from one container to another, precautions should be taken to avoid contamination with bacteria or with exogenous DNA.

Delayed engraftment of platelet lineage following stem cell transplantation
Isolated failure of platelet engraftment following stem cell transplantation can be due to the presence of pre-existing HPA specific antibodies in the recipient. These patients should be investigated in the same way as for platelet transfusion refractoriness.

Autoimmune thrombocytopenia
A raised level of platelet associated immunoglobulin (PAIg) is detected in the majority of patients with autoimmune thrombocytopenia (AITP). However the diagnostic specificity and therefore the clinical usefulness of the PAIg test by immunofluorescence is poor. Normally, these investigations are only indicated if the patient's platelet count is $< 100 \times 10^9$/L. The diagnostic specificity is increased if platelet glycoprotein specificity of the PAIgG can be determined by direct MAIPA assay but this assay requires a significant number of platelets which may be difficult to obtain from severely thrombocytopenic patients. The detection of serum platelet autoantibodies may be indicated, if the patient's platelets cannot be tested, but the results may be difficult to interpret because both alloantibodies and autoantibodies may be present in the serum.

These investigations are recommended only in the following categories of thrombocytopenic patients:

- Bone marrow failure possibly combined with immune-mediated thrombocytopenia
- AITP patients refractory to first and second line treatment
- Monoclonal gammaglobulinopathies
- Acquired autoantibody mediated thrombasthenia

Bone marrow failure and immune-mediated thrombocytopenia
In some patients with thrombocytopenia due to inadequate thrombocytopoiesis, antibody - mediated platelet destruction may compound the thrombocytopenia, e.g. patients with proliferative disorders such as chronic lymphocytic leukaemia (CLL) or stem cell transplant recipients. Reactive megakaryocytopoiesis is a diagnostic cornerstone of AITP but is not diagnostic if platelet autoimmunity is present in addition to bone marrow infiltration/failure. A PAIg test and determination of autoantibody specificity may be of use.

AITP patients refractory and first or second line treatment
For AITP patients for whom third line treatment is considered, a PAIg test, direct MAIPA assay and/or determination of antibody specificity may be indicated.

Monoclonal gammaglobulinopathies
Patients with a paraprotein in their serum (Monoclonal gammaglobulinopathies of unknown significance (MGUS), myeloma, secretory lymphoma) and a profound and unexplained thrombocytopenia should be investigated to determine whether the paraprotein is platelet reactive. Although rare, reactivity of paraproteins with platelets and thrombocytopenia has been reported and is the platelet equivalent of cold haemagglutinin disease.

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Drug-dependent immune thrombocytopenia (DDITP)
Many drugs are associated with thrombocytopenia. For some drugs there is firm evidence that the thrombocytopenia is antibody mediated. We recommend testing for DDITP for the following drugs:

- Heparin
- Antibiotics (penicillin type, beta-lactams and glycopeptide)
- Quinine and quinidine
- Gold salts

Heparin induced thrombocytopenia (HIT)
Heparin Induced Thrombocytopenia (HIT). An ELISA test for heparin/platelet factor 4 specific antibodies can be of use in patients with a clinical diagnosis of HIT and in whom continued anticoagulation is required. In such patients, prompt withdrawal of heparin and alternative anticoagulation with recombinant hirudin or an alternative heparinoid should be considered without waiting for laboratory results. The BCSH guidelines for the management of heparin induced thrombocytopenia[2] describe a scoring system (based on the **4T**s) that can be used to assess the probability of a patient developing HIT:

The 4Ts
- **T**hrombocytopenia
- **T**iming of platelet count fall
- **T**hrombosis
- O**T**her causes for thrombocytopenia are not evident

Table 9 should be used to assess the probability that a patient has HIT.
A score of: **6-8** means there is a high probability of HIT
4-5 means the probability is intermediate
0-3 means there is a low probability
If you think your patient has HIT, stop heparin and switch to an alternative antithrombotic agent.

Table 9: Assessment of the probability that a patient has HIT.

Estimating the probability of HIT: 'the **4T**s'			
Probability of HIT score:	Points (0, 1 or 2 for each of 4 categories: maximum possible score = 8)		
	2	**1**	**0**
Thrombocytopenia	>50% fall and/or platelet nadir 20-100 x 10^9/l	30-50% fall and/or platelet nadir 10-19 x 10^9/l	fall <30% and/or platelet nadir <10 x 10^9/l
Timing* of platelet count fall or other sequelae	Clear onset between days 5-10; or less than 1 day (if heparin exposure within past 100 days)	Consistent with immunisation but not clear (e.g. missing platelet counts) or onset of thrombocytopenia after day 10	Platelet count fall too early (without recent heparin exposure)
Thrombosis or other sequelae (e.g. skin lesions)	New thrombosis; skin necrosis; post heparin bolus acute systemic reaction	Progressive or recurrent thrombosis; erythematous skin lesions; suspected thrombosis not yet proven	None
O**T**her causes for thrombocytopenia are not evident	No other cause for platelet count fall is evident	Possible other cause is evident	Definite other cause is present

*First day of immunising heparin exposure considered day 0; the day the platelet count begins to fall is considered the day of onset of thrombocytopenia (it generally takes 1-3 days more until an arbitrary threshold that defines thrombocytopenia is passed).

[2] BCSH, Guidelines on the diagnosis and management of heparin induced thrombocytopenia: second edition – issue 2012, which can be found at: http://www.bcshguidelines.com/4_HAEMATOLOGY_GUIDELINES.html?dpage=1&sspage=0&ipage=0#gldocuments/HIT_2012.pdf

It is requested that the 4T test be applied to all patients for whom samples are referred for heparin dependent antibody investigation and the score entered on the request form. In selected circumstances additional information may be requested regarding some referrals.

In cases where patients have received a transfusion in the previous 12 days followed by a precipitous drop in platelet count, a diagnosis of post-transfusion purpura (PTP) should also be considered.

Other drugs
Investigation of the presence of platelet specific antibodies against other drugs is time consuming and positive control drug dependent antibody samples are typically not available. Reporting time will be extended since these investigations are not 'routine'. It is the referring centre's responsibility to provide samples of the implicated drug(s) (preferably in an aqueous form). Without such samples, the drug dependent antibody test will not proceed.

THROMBASTHENIA

Acquired thrombasthenia
Platelet autoantibodies generally target epitopes on GPIIb/IIIa (CD41), GPIb/IX/V (CD42), GPIa/IIa (CD49) or GPVI and, in some patients, the autoantibody may target the ligand binding site of these glycoproteins in cases with severe thrombocytopenia, a diagnosis of AITP is likely to be made. However, when the platelet count recovers during therapy a discrepancy between bleeding tendency and platelet count may be apparent. In such cases, platelet aggregation studies may be consistent with Glanzmann's thrombasthenia, Bernard Soulier syndrome or a collagen receptor deficiency of the acquired type. PAIg and autoantibody specificity investigations are important in these rare cases to confirm the true pathophysiology.

Glanzmann's thrombasthenia, Bernard Soulier syndrome, collagen receptor deficiencies
Homozygous or compound heterozygous mis-sense, non-sense mutations or deletions/insertions in genes encoding platelet membrane receptors can cause congenital bleeding disorders of the platelet type. Classic examples are Glanzmann's thrombasthenia (GT) and Bernard Soulier syndrome (BSS), which are both rare autosomal recessive disorders with an absence or reduced expression of the platelet αIIBß3 integrin (GPIIb/IIIa, CD61/41) and the Von Willebrand Factor receptor complex (GPIb/IX/V, CD42), respectively. Reduced expression of the platelet collagen receptors (GPIa/IIa or α2ß1 integrin and GPVI) have also been reported as a cause of congenital thrombasthenia.

Investigations
The diagnosis of GT, BSS and collagen receptor deficiencies is made on bleeding phenotype, by the results of platelet aggregation studies and, in classic BSS, on platelet count and morphology. However, mis-sense mutations associated with a mild phenotype might be missed in aggregation studies depending on the dose of agonist used and BSS without the striking morphology of giant platelets has been reported. Monoclonal platelet glycoprotein antibodies against CD41/61, CD42 and CD49 and flow cytometry provide a sensitive method to confirm the diagnosis. However, many laboratories only use a single monoclonal antibody for each CD marker, which limits the diagnostic sensitivity; null mutants will be identified but more subtle mis-sense mutations may remain undetected. Consequently, the patient's platelets are tested with a large panel of the relevant monoclonal antibodies to improve diagnostic sensitivity. These tests can only be performed after discussion with the H&I laboratory at Filton.

If the diagnosis of GT, BSS or inherited collagen receptor deficiency is confirmed, advice regarding transfusion support will be provided.

In addition direct sequencing of the coding regions of the relevant genes, BSS – *GPIbα, GPIbβ GP/IX* GT – *ITGA2B* and *ITGB3* is now available; please contact H&I laboratory at NHSBT Filton.

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CHAPTER 4C: GRANULOCYTE IMMUNOLOGY (GI)

There are seven clinical syndromes for which services are provided as shown in Table 10.

Table 10: Clinical syndromes involving granulocyte immunology (GI) services

• Autoimmune neutropenia
• Neonatal alloimmune neutropenia
• Severe and persistent non-haemolytic febrile transfusion reactions (see section 4A)
• Transfusion-related acute lung injury (see section 4A)
• Persistent isolated neutropenia after allogeneic bone marrow transplant
• Drug-induced antibody mediated neutropenia
• Severe reactions to granulocyte transfusions (see section 4A)

Autoimmune neutropenia
Autoimmune neutropenia (AIN) is a rare clinical condition caused by granulocyte autoantibodies, which may occur either in children or adults but which often remains undiagnosed. Autoimmune neutropenia commonly occurs in children between the ages of 6 months and 5 years (where it is referred to as autoimmune neutropenia of infancy - ANI, although applied strictly the term 'infancy' describes children under one year of age). ANI tends to be a self-limiting autoimmune condition but can last several years. In adult patients, AIN presents as a chronic disorder either as an isolated (primary) neutropenia or as a neutropenia secondary to other disorders, such as rheumatoid arthritis, systemic lupus erythematosus, Felty's syndrome and chronic lymphocytic and large granulocytic leukaemias.

Granulocyte autoantibodies may target the low affinity Fc receptor for IgG (FcγRIIIb or CD16); GP 56-64 kDa related antigens (CD177) or CD11/18. Autoantibodies can demonstrate HNA related specificity and therefore the sera are screened against a panel of granulocytes typed for the human neutrophil antigens (HNA). On occasion, it is important to determine whether antibodies with HNA specificity are autoimmune or alloimmune in origin. This can be achieved by typing the patient for the relevant HNA and/or performing a direct granulocyte immunofluorescence test. Immune complexes may also bind to granulocytes. There is no simple procedure to distinguish between immune complexes and pan-reactive autoantibodies.

Investigations
The serum will be investigated by the indirect granulocyte immunofluorescence and chemiluminescence tests using granulocytes from donors typed for HNA-1, -2, –3, 4 and 5. These investigations are only indicated if the patient has a neutrophil count < 2.0 x 10^9/L and the results will affect clinical management. Referrals without a stated neutrophil count or if the neutrophil count is >2.0x10^9/L or if inadequate clinical information is provided may not be investigated. If the serum test is negative, a direct granulocyte immunofluorescence test for IgG and IgM can be arranged with the laboratory but only in cases were there is strong evidence to support the diagnosis and where the result will influence clinical management. Elevated granulocyte bound immunoglobulins have been found in patients who lack demonstrable serum autoantibodies. Direct tests cannot be performed on patients with a neutrophil count < 0.4 x 10^9/L or if the patient has received G-CSF or IVIGG within the previous 3 weeks. Granulocytes are labile cells that deteriorate rapidly in vitro. Consequently, blood samples for direct tests must reach the GI section of the H&I laboratory at NHSBT Filton within 24 hours of venesection. The laboratory must be contacted prior to sending samples for direct tests so appropriate control samples can be arranged.

Neonatal alloimmune neutropenia
Neonatal alloimmune neutropenia (NAIN) is caused by maternal alloantibodies against a granulocyte-specific antigen, which is present on the neutrophils of the neonate and absent from the maternal neutrophils. The condition is rare (< 1 in 1000 births) but may be under-diagnosed. Profound neonatal neutropenia places the child at risk of infectious complications. The neutropenia may persist for up to

six months. In the majority of cases, the maternal alloantibodies are directed against HNA. Occasionally, NAIN can arise due to the formation of isoantibodies against granulocyte membrane glycoproteins, e.g. FcγRIIIb (CD16) which is absent in approximately 1 in 2000 of the population. Clinical management consists of the use of antibiotics either prophylactically or in response to infections. G-CSF may be required where there is severe persistent neutropenia and infection.

Investigations
Serum investigations are similar to those for autoimmunity, but a crossmatch of maternal serum versus paternal granulocytes may be performed to determine the presence of low frequency granulocyte-specific antibodies if initial investigations are negative. In the event of HLA specific antibodies being present, the serum sample will be further investigated by a glycoprotein capture ELISA (MAIGA assay). The maternal and paternal HNA type will be determined. In serologically confirmed cases of NAIN involving HNA specific antibodies, the zygosity of the father of the child should be determined so that the risk to future pregnancies can be assessed.

Persistent isolated neutropenia after bone marrow transplant
Both HNA alloantibodies and autoantibodies can cause persistent isolated neutropenia after bone marrow transplantation. Granulocyte immunology investigations can be informative in such cases. Investigations are similar to those described above.

Drug-induced antibody mediated neutropenia
A wide range of drugs can cause immune mediated neutropenia. However, these idiosyncratic reactions only occur in a small number of patients. There are several mechanisms for drug induced antibody mediated neutropenia. One established mechanism occurs when membrane glycoproteins bind to the drug to form a hapten. This causes the formation of antibodies which only bind to granulocytes in the presence of the drug. Quinine, and its stereoisomer quinidine, is known to cause drug dependent antibody formation via this hapten mechanism.

Other drugs (e.g. ß-lactams) have been reported to elicit the formation of antibodies. Alternatively, some drugs induce the formation of 'true' autoantibodies, which are able to bind granulocytes in the absence of any drug. These drugs (e.g. levamisole) appear to alter the homeostasis of the immune system resulting in autoimmunity against granulocytes in a small number of patients.

The investigation of cases with drug dependent antibodies can be complicated. Furthermore, some antibodies have been reported to only be detected at specific concentrations of the drug, by specific techniques or in the presence of drug metabolites. Please phone the H&I laboratory at NHSBT Filton before referring such cases.

Investigations
The patient serum sample is investigated for granulocyte-specific antibodies by granulocyte immunofluorescence tests using a panel of granulocytes typed for HNA-1 to -5 in the presence and absence of the implicated drug. The referring centre must provide a sample of the implicated drug(s). Further investigations may require the provision of anti-coagulated blood from the patient.

Granulocyte transfusion reactions
An increment in granulocyte count greater than 0.5×10^9/L is not always achieved in profoundly granulocytopenic recipients by granulocyte transfusions. An incremental count would be expected to be seen with granulocyte doses of at least $1x10^{10}$ granulocytes/m^2 of recipient surface area. Severe reactions to granulocyte transfusions and failure to increment despite adequate granulocyte dosage may suggest HLA or granulocyte specific antibody formation in the recipient and in these cases referral for antibody screening is advised. The investigations are similar to those described previously and where necessary HNA typing of the patient and implicated and/or prospective donors will be undertaken.

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CHAPTER 4D: HAEMATOPOIETIC STEM CELL TRANSPLANTATION

HLA typing of recipients and related or unrelated donors
Incompatibility in the HLA expressed by the recipient and the stem cell donor is one of the most important factors influencing the outcome of transplantation. It is therefore crucial that the most up-to-date techniques are used to identify these incompatibilities at the DNA level. NHSBT H&I laboratories are perfectly placed to carry out these tests since a significant number of patients prepared for haematopoietic stem cell transplant are also investigated by NHSBT for their platelet transfusion support. All aspects of the service are compliant with the relevant standards for haematopoietic stem cell transplantation specifically:

- Standards for Histocompatibility Testing Version 6.1 European Federation for Immunogenetics (EFI) October 2013

HLA antibody screening for haematopoietic stem cell transplant patients
For certain patients undergoing allogeneic stem cell transplantation it is advisable to perform HLA class I (and class II) antibody screening well in advance. As the use of alternative donors (e.g. HLA mismatched adult donors, haploidentical donors and cord blood) for HSCT is increasing the relevance of HLA specific antibodies on donor compatibility becomes critical. Knowledge of the patient's antibody status is of value when selecting the final donor for transplant and assessing overall risk. Platelet transfusion support may also be complicated in HLA antibody positive patients.

Unrelated donor searches of the stem cell and cord blood registries
NHSBT provides a facility for searching national and international unrelated stem cell and cord blood registries for patients requiring haematopoietic stem cell transplantation where no HLA compatible family member has been identified. Requests from transplant centres for searches of registries should be made via the local NHSBT H&I laboratory. A search via NHSBT H&I laboratories will automatically be referred to the Anthony Nolan Trust (ANT) who, on behalf of the aligned registries, will undertake a search of volunteer unrelated donors held on the British Bone Marrow Registry (BBMR), Welsh Bone Marrow Donor registry (WBMDR) and Anthony Nolan Registry. When required, searching of international registries can also be initiated. For cord blood stem cells, the UK cord blood registry and other international cord blood registries are searched. The H&I laboratory will co-ordinate the donor searches and the request for confirmatory typing of potential donors. They will also advise on the final selection of the most suitable donor and will liaise on behalf of the requester with relevant donor registry.

Graft information advisory service (GIAS) + compatibility assessment
Graft Information Advisory Service (GIAS) and compatibility assessment is an integral part of haematopoietic stem cell transplantation support from our laboratories. Our services are always supported by the highest standards of advice from Consultant Clinical Scientists and their staff. Clinical Scientist staff will support the identification and selection of donors most advantageous to your patients. This encompasses the whole process from diagnosis to transplant and beyond with transplant and antibody monitoring and supply of specialised selected blood products when needed.

Chimerism investigation for post transplant monitoring

Fluorescently labelled PCR primers are used to amplify STR loci resulting in an 'STR profile' for the patient pre-transplant, the donor and the patient post-transplant. This allows assessment of the chimeric status of the patient following transplantation.

Pre transplant sample from the patient and an EDTA blood sample or bone marrow aspirate post-transplant is required for STR analysis. A donor sample is also required. Where possible, if sample size allows, both patient pre-transplant and donor DNA will be stored at laboratories where HLA typing has been performed. If stored sample is not available it is possible to isolate DNA from the buccal

cells of the patient, with the resulting DNA being the equivalent of a pre-transplant sample. Your local H&I laboratory will be able to advise.

Data has demonstrated that increased sensitivity can be achieved in the investigation of chimerism when isolating specific cell lineages e.g. T cells. This may be particularly relevant for patients with certain malignancies, where cell lineage isolation prior to STR analysis can detect changes in chimeric status otherwise undetectable by whole blood analysis. NHSBT H&I laboratories are able to perform STR analysis on specific cell lineages, e.g. T cells, the myeloid compartment and B cells. Again, an EDTA blood sample from the patient post-transplant is required for this analysis.

STR analysis is also a critical diagnostic tool in the investigation of transfusion-associated graft versus host disease (TAGVHD). STR profiles can be established for the patient pre-transfusion, the implicated donor and the patient post-transfusion. This allows assessment of the chimeric status of the patient post-transfusion. Samples required for this analysis would be a patient pre-transfusion sample (if no DNA has previously been isolated from this patient, then a buccal scrape would provide cells for DNA isolation), an EDTA blood sample from the donor and an EDTA sample from the patient post-transfusion.

CHAPTER 4E: SOLID ORGAN TRANSPLANTATION

The H&I laboratories support organ transplantation (SOTx) by identifying and characterising immunological risk factors that determine outcome and provide advice accordingly. These risk factors are the degree of human leucocyte antigen (HLA) mismatch between donor and recipient and specific sensitisation to non-self HLA. A 24 hour on-call service operates every day of the year for deceased donor HLA typing and crossmatching of local patients for renal and, where appropriate, cardiothoracic transplantation. Our aim is to work in partnership with the transplant and clinical units as part of the overall transplant team. Accountability for service provision, development and governance lies with the local H&I Consultant. Development of local transplant policies, particularly allocation rules should include liaison with the Head of Laboratory. Clinical and scientific advice from an H&I Consultant Clinical Scientist relating to solid organ transplantation is also always available.

HLA typing for SOTx

In the UK, deceased donor kidneys are currently allocated though NHSBT Organ Donation & Transplantation (ODT) using matching algorithms in which HLA match is a key factor. Thus all patients are required to be HLA typed before being placed on the transplant waiting list. Donors will be HLA typed and then allocated to recipients on the list based on factors including HLA match, the matching schemes for organ allocation can be found on the ODT web site at: http://www.organdonation.nhs.uk/about_transplants/organ_allocation/.

Because of the extreme variability of HLA in the population, most patients will receive a graft from a donor mismatched to some degree for HLA. The greater the degree of mismatch the greater risk of immunological rejection, however, by modifying the immunosuppression this may be compensated. HLA matching is normally not a primary consideration in other forms of transplantation (cardiothoracic, liver, etc). Graft failure is often associated with immunological sensitisation to mismatched donor HLA and this can severely limit the possibility of retransplantation if this were to be an option. It is the responsibility of the clinical teams to inform the laboratory if a patient has been exposed to a specific sensitisation event and provide a test sample.

Routine testing for HLA-specific antibodies

HLA-specific sensitisation is best investigated by serological analysis for antibodies. Any exposure to non-self HLA, such as from transplantation, transfusion or pregnancy can stimulate the production of HLA specific antibodies. These can vary in their potency and persistence depending on the nature and number of stimulating events but represent a significant risk of graft failure. All patients on a transplant waiting list should therefore be monitored regularly for the presence of HLA specific

antibodies. For prospective kidney and cardiothoracic transplant patients the recommendation is that each patient should be tested at least three monthly and after each potential sensitising event[3].

All antibody positive sera will be characterised for specificity for all known HLA A, B, C, DR, DP and DQ antigens. For some sera (i.e. those from highly sensitised patients, reacting with over 80% of the donor population) this may require successive testing by increasingly sensitive and specific techniques. In such cases the completion of testing may take significantly longer than for less complex cases.

For certain highly sensitised patients pre-transplant antibody removal (desensitisation) may offer the only possibility of being transplanted. NHSBT H&I laboratories can support such procedures, but because it is excessively labour-intensive for the laboratory this must be discussed and planned with the Head of the Laboratory before proceeding.

Post transplant antibody monitoring is recommended for most types of solid organ transplantation[2]. For immunologically high risk transplants antibody monitoring should be intensified. For any transplant, if rejection is suspected, a test for donor specific antibodies can confirm a diagnosis of rejection and indicate a course of management. Such testing can be performed on demand but usually only during normal working hours.

Crossmatching

If present at a high concentration, patient antibodies corresponding to mismatched donor HLA can cause immediate and irreversible rejection of the transplanted organ. The presence of donor HLA specific antibodies in the serum of the patient at any time prior to transplant is an indication of prior sensitisation and even in cases where these antibodies are not present at a high concentration at the time of transplant they indicate there may be an increased risk of accelerated acute or acute rejection. Performing a prospective crossmatch between donor and recipient can prevent hyperacute rejection and identify some patients at risk of acute rejection. A pretransplant crossmatch can therefore avoid an unintentional antibody incompatible transplant and is performed in one of two ways. Firstly, using donor cells (peripheral blood leucocytes or leucocytes obtained from spleen or lymph nodes) donor reactive antibodies can be assessed directly by either CDC (complement-dependent cytotoxicity) or flow cytometry; the latter being a more sensitive assay. Secondly, using the results of HLA antibody specificity tests on the recipient together with the HLA type of the donor a virtual crossmatch (VXM) can be performed. Essentially the VXM predicts the result of a donor cell-based crossmatch and is dependent on a comprehensive knowledge of the specificity of any detected antibody and its potential reactivity with a donor of given HLA type. Virtual crossmatching is routinely used in cardiothoracic transplantation where time does not allow for a cell based crossmatch to be completed. In renal transplantation virtual crossmatching may be used for a well defined population of potential recipients but is not currently recommended for highly sensitised patients.

A prospective crossmatch is required or recommended in renal, pancreatic, cardiothoracic and small bowel transplantation. The choice of pretransplant crossmatch can vary with transplant type and should be controlled by local policies guided by national policies, guidelines and accreditation standards. Where the prospective crossmatch was a virtual crossmatch a retrospective donor leucocyte confirmatory crossmatch should always be done.

The time taken to perform the leucocyte crossmatch is usually between 3 and 6 hours of laboratory time. A VXM can usually be completed in 30 minutes but this does require the on-call H&I scientist to go to the laboratory in order to review the patient's serological history.

The results of crossmatch tests can be highly complex, particularly in patients with historically high levels of antibodies which have since decreased. Specialised interpretation of these results is necessary to determine their clinical significance. Advice on specific cases will be provided by the H&I Consultant Clinical Scientist, as required.

[3] BTS/BSHI Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation, 2010, which can be found at: http://www.bshi.org.uk/html/guidelines.html

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Pretransplant antibody removal (Desensitisation)
Pretransplant antibody removal, undertaken to allow transplantation in crossmatch positive cases (Antibody incompatible Transplantation, AiT) is termed desensitisation. Incompatibility is either due to ABO mismatch or preformed HLA donor specific antibodies (DSA). Desensitisation is achieved by extracorporeal antibody removal using various techniques.

During the desensitisation process antibody removal should be monitored so that the effectiveness of the process can be assessed and a safe level of residual antibody can be determined before the transplant can proceed. During the early post-transplant phase DSA can be re-synthesised and cause rejection. Early detection of an emerging response allows effective treatment and management of rejection. Frequent DSA monitoring with fast turn around times are therefore essential for a safe desensitisation programme.

Rapid DSA testing requires significant resources and scientific staff need to be available on demand. Therefore if laboratory support for AiT is required there must be a formal agreement with the laboratory to allocate the necessary resources. Effective communication between the laboratory and the transplant unit is essential for these high risk procedures to be undertaken with safety. A reliable and unimpeded sample transport system must be established.

Tests:
ABO AiT: Antibody levels will be monitored in terms of titre of IgG and IgM using reagent red cells of the donor group. Transplantation would not normally proceed if the titre of the corresponding antibody exceeds 1:8. It is therefore very important to test immediately before the transplant is to proceed. In NHSBT ABO testing is carried out by Red Cell Immunohematology (RCI) who will make a separate charge for this service.

HLA AiT: The amount of desensitisation required will depend on the pre-treatment levels of DSA. The highest levels of DSA will be cytotoxic and a cytotoxic titre assay should be performed to measure these against donor lymphocytes (CDC crossmatch). The strength of non-cytotoxic DSA should be assessed by a flow cytometric crossmatch (FCXM).

DSA levels and specificity are most effectively monitored using antigen coated beads in an immunofluorescence assay (e.g. Luminex ™). These assays are significantly more sensitive than previous methods and our experience shows that reducing DSA to undetectable by desensitisation is rarely achieved. An assessment of a safe level for transplantation needs to be determined for each case by discussing with the H&I Consultant. A pre-transplant crossmatch should always be performed, normally a FCXM is sufficient.

Frequency of testing
During antibody removal, pre- and post-treatment serum samples should be sent directly to the laboratory. Throughout the early post transplant phase (up to three weeks) daily serum samples should be taken and sent to the laboratory. Early post-transplant antibody resynthesis can be treated with antibody removal if accompanied by rejection and this should be monitored as above. From week three approximately weekly serum samples should be taken for antibody testing until a stable antibody profile is established (usually 3-5 months). Thereafter monthly samples should be taken to year 1 followed by six-monthly samples.

Additional Services
In addition to performing and reporting tests, there are certain supporting and administrative elements provided by the laboratory, which may constitute part of the H&I service required for a transplant programme. It is important that close liaison is maintained between the laboratory and the clinical transplant units in order to establish good working relationships with the medical and nursing staff. Senior laboratory staff should attend relevant clinical and audit meetings. The H&I laboratory should play a major role within the multidisciplinary team involved in the provision, planning and development of clinical transplantation services.

The laboratory maintains a database of successive test results for all patients and their donors. From this we can establish and review each patient's immunological history and where necessary provide advice on general transplant suitability and specific advice regarding risk of individual transplants.

For patients on the national renal transplant waiting list (at Organ Donation and Transplantation (ODT)), the H&I laboratory will be responsible, if required, for updating the ODT database with HLA typing and antibody data and collating other information as requested. In addition, registration of new patients with ODT can be performed by the H&I laboratory. To do this, additional information, such as demographics, virology status, and blood group must be given to the laboratory.

Blood grouping of donors and recipients can be undertaken by NHSBT RCI samples should be sent to your local NHSBT RCI department with a test request form alternatively samples and request forms can be forwarded to RCI by your local H&I laboratory although this may contribute to the turnaround time of the test, an additional charge will be made for this grouping service by RCI.

Further information about NHSBT RCI services can be found in their user guide at: http://hospital.blood.co.uk/library/user_guides/index.asp.

Where a local transplant waiting list is required this can also be maintained and distributed by the laboratory. The laboratory database has the functionality to identify patients from whom we have not received sufficiently recent samples. We can send written reminders to the clinical units, as part of our service. Failure to provide up-to-date serum samples can compromise the chance of a patient receiving a transplant.

Out of hours
Out of hours on-call is provided by H&I laboratories supporting solid organ transplant programmes, 24 hours a day, 365 days a year. A Consultant Clinical Scientist can be contacted on your request in case of clinical emergency via your local Hospital Services department.

CHAPTER 4F: IMMUNOGENETICS - HLA TYPING FOR DISEASE ASSOCIATION AND DRUG HYPERSENSITIVITY

Genetic variations (mutations or polymorphism) within genes are now known to occur frequently throughout the human genome. Amongst these are mutations in genes located on or in proximity to the major histocompatibility locus (MHC) on the short arm of chromosome 6. Genetic markers determining the risk for the development of certain diseases can be identified by testing performed by the NHSBT H&I laboratories.

Examples of HLA genes associated with disease include HLA-B27 with ankylosing spondylitis and specific HLA-DQ genes with coeliac disease (CD). In CD only certain HLA-DQ heterodimers are able to present the gluten peptides to immune cells and initiate the response which leads to CD. Complete testing of both DQ alpha and DQ beta genes is required in order to identify the implicated alleles.

The HFE gene associated with hereditary haemochromatosis is another gene found in the MHC region. Two mis-sense mutations in the HFE gene, a cysteine282tyrosine and a histidine63aspartic acid, have both been shown to be associated with the development of disease. Between 80-90% of haemochromatosis cases are homozygous for the tyrosine282 codon. In addition, up to 78% of individuals heterozygous for both mutations may exhibit evidence of iron overload. A DNA based technique that allows the simultaneous identification of both HFE mutations has been developed and validated by participation in UKNEQAS.

HLA genes have also been found to be markers of some drug hypersensitivity responses. HLA-B*57:01 is associated with hypersenitivity to the anti-retroviral agent Abacavir. The H&I laboratories routinely provide HLA-B*57:01 testing and testing for other HLA alleles implicated in drug

hypersensitivity reactions can also be provided on request. In addition tests for the detection of mutation in genes involved in the metabolism and absorption of immunosuppressive drugs are being developed and may be offered by the H&I laboratories supporting solid organ transplant programmes. All mutations are defined by using molecular, DNA-based typing techniques.

In addition to identifying HLA polymorphism, H&I laboratories also provide molecular typing for detecting mutations in other immune related genes such as minor histocompatibility (mH) genes, natural killer (NK) cell receptor genes and cytokine genes.

Examples of HLA associated and HLA linked diseases are shown below. There are additional HLA associated diseases for which a typing service can be provided. Please contact the Head of Laboratory of your local NHSBT H&I laboratory to discuss the appropriate test.

Genetic markers and diseases

1. HLA associated diseases

Birdshot chorioretinopathy	HLA-A29
Bechet's disease	HLA- B51
Ankylosing spondylitis	HLA-B27
Rheumatoid arthritis	Amino acids 70-74 on the DRB1 gene (QKRAA or QRRAA)
Narcolepsy	HLA-DQB1*06:02/DQA1*01:02
Coeliac disease	HLA-DQA1/DQB1
Selective IgA deficiency	HLA-DRB1*0301/DQB1*02
Development of anti-HPA-1a in NAIT*	HLA-DRB3*01:01

* NAIT, neonatal alloimmune thrombocytopenia

2. HLA linked diseases

Haemochromatosis	HFE gene C282Y and H63D
21 OH deficiency	(HLA-B47) 21 OH gene

3. HLA genes associated with drug hypersensitivity / Adverse Drug Reactions (ADR)

Abacavir hypersensitivity	HLA-B*57:01
Allopurinol induced severe cutaneous adverse reactions	HLA-B*58:01
Carbamazepine induced Stevens-Johnson Syndrome / Toxic Epidermal Necrosis (SJS/TEN)	HLA-B*15:02 (Han Chinese / Thai) HLA*A31:01 (Japanese / Caucasian)

Please enquire for other testing you may require.

4. Polymorphism in other immune-related genes

Cytokines and cytokine receptor genes	e.g. *TNFA*, *IL-10* and *IL-6*
NK cell receptors	
Minor histocompatibility antigens	(HA-1)

CHAPTER 5: TECHNIQUES USED IN NHSBT H&I LABORATORIES

The following techniques are routinely used in the H&I laboratories, although further techniques are also used for the investigation of suspected thrombasthenias and further studies. Please refer to the appropriate section in this guide for details regarding techniques used in specific clinical conditions.

Screening for cytotoxic and non-cytotoxic HLA specific antibodies
Screening for HLA class I and class II specific antibodies is performed using one or more of a number of different techniques including the classic microlymphocytotoxicity test, an ELISA based technique, Luminex ™ and flow cytometric based methods. If the screening is positive, further tests are carried out to identify the specificity of the antibodies.

Molecular HLA class I and class II typing
HLA class I (A, B, C) and class II (DR, DQ, DP) DNA typing is carried out using a variety of DNA based techniques including sequence specific priming (SSP) and sequence specific oligonucleotide probing (SSOP). Furthermore, HLA class I and II high resolution typing used to provide an HLA type at the allele level for bone marrow, peripheral blood or cord blood stem cell transplant patients and their related or unrelated donors is done using DNA Sequence Based Typing (SBT). All molecular techniques used for HLA class I and class II molecular typing have been fully validated as part of the participation in national and international histocompatibility workshops and quality assurance schemes.

Crossmatching
Different techniques are used by the laboratories which includes microlymphocytotoxicity test and flow cytometric based methods.

Short Tandem Repeat (STR) analysis for the detection of chimerism

Fluorescently labelled PCR primers are used to amplify STR loci resulting in an ‘STR profile’ for the patient pre-transplant, the donor and the patient post-transplant. This allows assessment of the chimeric status of the patient following transplantation or during investigation of suspected TaGVHD. NHSBT H&I laboratories are able to perform STR analysis on specific cell lineages, e.g. T cells, the myeloid compartment and B cells

Screening for platelet specific antibodies
The Platelet Immunofluorescence Test (PIFT) with a flow cytometric endpoint and the Monoclonal Antibody Immobilisation of Platelet Antigen (MAIPA) assay together with a panel of HPA typed platelets are used to facilitate the detection and identification of antibodies directed against platelet membrane glycoproteins. Both techniques are usually performed as an indirect test using patient serum but both tests can also used as a direct test to detect immunoglobulins bound to patient’s platelets and identify platelet glycoprotein specificities. Typically the direct MAIPA assay is only performed for specific patients after discussion with the H&I laboratory at NHSBT Filton.

Molecular typing for HPA alleles (1, 2, 3, 4, 5, 6, 9 and 15)
Determination of HPA alleles (1, 2, 3, 4, 5, 6, 9 and 15) is performed using sequence based typing (PCRSBT).

Heparin Induced Thrombocytopenia
ELISA tests for heparin dependent platelet factor 4 specific antibodies are performed in suspected cases of Heparin Induced Thrombocytopenia (HIT). Excess heparin is added to the test system to confirm that positive reactions are heparin dependent rather than autoantibodies. Additional testing using different ELISA assays is available for use in specific cases where initial test results are ambiguous.

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Screening for granulocyte specific antibodies

The granulocyte immunofluorescence test (GIFT) with a flow cytometric endpoint, the granulocyte chemiluminescence test (GCLT) and the Monoclonal Antibody Immobilisation of Granulocyte Antigen (MAIGA) assay are used together with a HNA typed granulocyte panel to facilitate the detection and identification of antibodies directed against granulocyte membrane glycoproteins. These techniques are usually performed as indirect tests using patient serum. Direct immunofluorescence tests using the patient's granulocytes can be performed in certain cases but these investigations are restricted by the patient's neutrophil count and the necessity to test the samples within 24 hours of venesection. A direct test will only be performed after tests for granulocyte serum antibodies have been performed and must be arranged in advance with the H&I laboratory at NHSBT Filton. Granulocyte Immunology investigations can be prolonged compared to other investigations because granulocytes are labile cells and cannot be stored for testing.

Typing for HNA

HNA (-1, -2, -3, -4 and -5) are typically performed either by serology (HNA-2) or polymerase chain reaction by sequence based typing (PCRSBT).HNA-1, -3, -4, -5).

CHAPTER 6: ORDERING SPECIALIST PRODUCTS

Ordering blood products
All standard blood products are ordered from NHSBT Hospital Services, which can be contacted 24 hours per day, each day of the year. Use direct dial numbers during normal working hours and for out of hours. Medical and scientific advice is available 24 hours a day. Refer to chapter 7 for contact details.

Specialist products issued by the H&I function
HLA selected platelets are ordered directly from your local NHSBT H&I laboratory during laboratory operating hours (9:00 – 5:00). This is also the case, for platelet refractory patients with both HLA & HPA specific antibodies and HPA specific antibodies only. The laboratory staff can be contacted directly and will liaise on your behalf with the respective NHSBT Hospital Services and Transport departments to organise delivery either directly to your hospital or via your local blood centre.

Ordering of HLA (or HPA & HLA) selected platelets
To order HLA (or HLA & HPA) selected platelets, in the first instance, contact your local NHSBT H&I laboratory. Details of the ordering process can be found on the NHSBT Hospital and Science website at: http://hospital.blood.co.uk/library/request_forms/hla/order_hla/.

Order notice time
It is recommended that orders are placed with sufficient time for the best available product to be selected for your patient. Orders received at short notice (<24 hours) may result in units being selected only from nearby NHSBT facilities to allow delivery in the time available, possibly excluding units that would lead to a better outcome for your patient. Your co-operation in this matter is appreciated.

When an order is placed for the first time for a patient the following information is required:

- Patient surname, first name, date of birth and hospital name in full
- NHS number
- ABO and D groups
- HLA class I type (if known)
- Clinical diagnosis (Bleeding grade if known)
- CMV antibody status of patient
- Period of expected thrombocytopenia
- Contact person at hospital transfusion laboratory
- Consultant or Specialist Registrar responsible
- Current platelet support
- Patient weight

In case of an allogeneic stem cell transplant, the following information is required for the donor:

- CMV antibody status
- HLA class I type (if known)
- ABO and D groups

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Ordering of HPA selected platelets and red cells (non-refractory)
All orders for HPA selected platelets and red cells for non-refractory patients, during normal working hours should be made directly to the H&I laboratory at NHSBT Filton. When an order is placed for the first time for a patient the following information is required:

- Patient surname, first name, date of birth and hospital number or patient address
- NHS number
- Hospital name
- ABO and D groups
- Contact person at hospital transfusion laboratory
- Consultant or specialist registrar responsible
- Clinical diagnosis
- Red cell specific antibodies present
- Type, quantity of product required, date and time needed

HPA typed products
The following HPA typed products are available:

- HPA-1a negative red cells
- HPA-1a and HPA-5b negative apheresis platelet concentrates for neonates (neonatal dose)
- HPA-1a or HPA-5b negative platelet hyperconcentrates for foetal use from accredited donors#
- Red cells or platelet concentrates typed for other HPA antigens *

These must be ordered at least 7 days in advance
* These products may not be available 'off the shelf'

HPA-1a and 5b negative typed platelet concentrates and red cells are banked at a limited number of blood centres. These products are ordered during working hours from the H&I laboratory at NHSBT Filton. During 'out of hours', contact the Hospital Services department at your local blood centre.

HPA-1a negative red cell SAG-M concentrates and HPA-1a and HPA-5b negative apheresis platelet concentrates (neonatal dose, 1/4 of a standard adult dose) are normally available 'off the shelf' at selected centres. Apheresis platelet concentrates or red cells negative for other HPA antigens need to be ordered well in advance (Ideally at least 4-7 working days). Additional HPA typing can be performed on request, please discuss with the H&I laboratory at NHSBT Filton.

HPA-1a negative platelet hyperconcentrates for use are provided from specially accredited apheresis donors who lack antibodies against red cells, HLA or HPA and are CMV negative. The first hyperconcentrate needs to be obtained from an RhD negative donor before the type of foetus is determined. Please contact the laboratory in advance (Ideally at least 7 working days) for hyperconcentrates as this product has a shelf life of 24 hours and is not a stock item. A request form for the ordering of platelet hyperconcentrates is available from the H&I laboratory at NHSBT Filton. The laboratory must be informed on pre- and post- transfusion platelet counts to ensure the effectiveness of the treatment.

Out of hours
Selected platelets should be ordered during normal working hours and special attention must be paid to planning and ordering platelets required over weekends and bank holidays in advance. An out of hours service is available for **unexpected clinically urgent cases** only. For H&I related advice on clinically urgent cases out of hours then phone NHSBT Hospital Services at your local centre. They can put you in contact with the on call H&I Consultant Clinical Scientist.
For further details refer to: http://hospital.blood.co.uk/library/request_forms/hla/order_hla/

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H&I platelet immunology services do not provide a laboratory 'out of hours' service. However HPA -1a(-), 5b(-) typed platelets can be issued from stock on request. Requests for HPA selected neonatal platelets and HPA selected red cells, should be discussed with a NHSBT Medical Consultant. Phone the NHSBT Hospital Services and they can put you in contact with the 'on call' Medical Consultant. For further details refer to: http://hospital.blood.co.uk/library/request_forms/hla/order_hpa/.

CHAPTER 7: COMMUNICATING WITH NHSBT CENTRES

NHSBT staff can be contacted by telephone, facsimile or e-mail either directly using their personal details or through the centre switchboards.

By direct dialling
All departments and senior staff can be contacted directly using direct dial numbers in this guide. Our internal telephone system allows external calls to be transferred readily between departments and between centres and to mobile phones.

Via switchboard
Alternatively, all centres can be contacted 24 hours per day, 7 days a week via the switchboard number during office hours and via NHSBT Hospital services outside office hours.

Via mobile phone
Mobile phones are used by the majority of Medical & Clinical Scientist Consultants, senior scientific and managerial staff. External calls to any of the blood centres in the country can be directly transferred to the mobile phones. The secretariats can advise on how to contact a member of staff when he/she is not at his/her base centre.

Sending a fax
All centres have central fax facilities. It is therefore important that your fax is labelled clearly with the name of the person to who you wish to send it and if urgent, please indicate accordingly. Nearly all H&I laboratories and all Hospital Services departments have their own fax.

Via e-mail
All H&I staff listed can be contacted by e-mail using the following address format:
firstname.surname@nhsbt.nhs.uk, Alternatively, please use hinational@nhsbt.nhs.uk for generic enquiries.

For safety reasons attachments with incoming e-mails will be scanned and can be placed in quarantine. The sender and the addressee will be informed automatically when this safety mechanism is triggered.

NBSBT maintains several websites including:
http://www.nhsbt.nhs.uk/,
www.blood.co.uk for donors and
http://hospital.blood.co.uk/index.asp for healthcare professionals where information regarding all aspects blood donation, blood stock levels and information about services can be found.

Customer Services
If you have a query regarding the services provided by NHSBT you can also contact one of our Customer Services Managers. Each centre has a Customer Services Manager who works closely with local consultants and scientists. The Customer Services Managers are responsible for understanding the requirements of service users and for acting as a central point for contacts for technical, operational and financial issues. For contact details refer to: http://hospital.blood.co.uk/contact_us/.

How to enrol as a donor
Should you wish to enrol as a donor or want information on blood or platelet donation and donation session times please contact NHSBT national donor call-centre on 0300 123 2323, open 24 hours per day, 7 days per week or visit our website www.blood.co.uk or download our session searcher app for smartphones and tablets (available for both android and apple) at: http://www.blood.co.uk/giving-blood/where-can-i-go/smartphone-apps/.

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H&I HEADS OF LABORATORIES CONTACT DETAILS

Title	Name	Surname	Role	Location	Regional code	Local code	Fnet
Dr	Andrea	Harmer	National Head of H&I	Sheffield	0114	358	4914
	Debra	Marples	PA	Sheffield	0114	358	4935
Prof.	David	Briggs	Head of Laboratory	Birmingham	0121	278	4099
	Pauline	Hall	PA	Birmingham	0121	278	4109
Dr	Colin	Brown	Head of Laboratory	Colindale	020	8957	2811
	Usha	Mistry	PA	Colindale	020	8957	2824
Dr	Anthony	Poles	Head of Laboratory	Filton	0117	921	7533
	Tuarita	Lawson	PA	Filton	0117	921	7478
	Tim	Key	Head of Laboratory	Sheffield	0114	358	4876
	Claire	Mcfarlane	PA	Sheffield	0114	358	4935
Dr	Martin	Howell	Head of Laboratory	Newcastle	0191	202	4475
	Alison	Campbell	PA	Newcastle	0191	202	4558
Dr	Deborah	Sage	Head of Laboratory	Tooting	020	3123	8567
	Jackie	Davis	PA	Tooting	020	3123	8387

LABORATORY CONTACTS DETAILS

H&I service	Centre	Contact name			Lab	Selected products	Fax
H&I	Birmingham	David Briggs	0121 278	4099	4105/ 4108	4196	4102
H&I/PI/GI	Filton	Anthony Poles	0117 921 0117 912	7473	5733	5728	5731
H&I	Colindale	Colin Brown	020 8957	2811	2812	2819	2973
H&I	Newcastle	Martin Howell	0191 202	4475	4410	4525	4564
H&I	Sheffield	Tim Key	0114 358	4914	4839 / 4830	4806	4850
H&I	Tooting	Deborah Sage	020 3123	8567	8347	8488	8486

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BLOOD CENTRE DETAILS

			Switchboard		Hospital Services	
Centre	Address	Postcode	Telephone	FAX	Telephone	FAX
Birmingham	**Vincent Drive, Edgbaston, Birmingham**	**B15 2SG**	**0121 278 4000**	**4005**	**4037**	**4039**
Brentwood	Crescent Drive, Brentwood, Essex	CM15 8DP	01277 72 1000	1032	1005	1128
Filton	**500 North Bristol Park, Northway, Filton, Bristol**	**BS34 7QH**	**0117 912 0117 921 7200**	**7201**	**5724**	**5783**
Cambridge	Long road, Cambridge	CB2 0PT	01223 58 8000	8114	8021	8121
Colindale	**Colindale, Charcot Road, London**	**NW9 5BG**	**020 8957 2700**	**2970**	**2800**	**2971**
Lancaster	Ashton Road, Lancaster	LA1 4GT	01524 89 6220	6222		
Leeds	Bridle Path, Leeds	LS15 7TW	0113 820 8600	8737	8607	8738
Liverpool	14 Estuary Banks , Speke, Liverpool	L24 8RB	0151 268 7000	7001	7170	7173
Manchester	Plymouth Grove, Manchester	M13 9LL	0161 423 4200	4245	4201	4358
Newcastle	**Holland Drive, Newcastle upon Tyne**	**NE2 4NQ**	**0191 202 4400**	**4505**	**4500**	**4514**
Oxford	John Radcliffe Hospital, Headington, Oxford	OX3 9BQ	01865 38 7900	7915	7963	7997
Plymouth	Derriford Hospital, Derriford Road, Plymouth	PL6 8DH	01752 63 7815	7816	7802	7810
Sheffield	**Longley Lane, Sheffield**	**S5 7JN**	**0114 358 4800**	**4911**	**4817**	**4952**
Southampton	Coxford Road, Southampton	SO16 5AF	023 8035 6700	6760	6712	2060
Tooting	**75 Cranmer Terrace, London**	**SW17 ORB**	**020 3123 8300**	**8453**	**8352**	**8449**

Bold type indicates centres with H&I laboratories

		PHONE	FAX
British Bone Marrow Registry, NHSBT Filton, 500 North Bristol Park, Northway, Filton, Bristol			
Organ Donation and Transplantation (ODT) Fox Den Road, Stoke Gifford, Bristol	BS34 8RR	0117 975 7575	0117 975 7577

User Guide for Histocompatibility and Immunogenetics Diagnostics Services

CHAPTER 8: STANDARDS, GUIDELINES & ACRONYMS

British Committee for Standards in Haematology guidelines
http://www.bcshguidelines.com/4_HAEMATOLOGY_GUIDELINES.html

Guidance on the microbiological safety of human organs, tissues and cells used in transplantation (2011) British Transplantation Society
http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_121497

Guidelines for the Blood Transfusion Services in the United Kingdom 8th edition (2013)
The Stationery Office, London, UK
http://www.transfusionguidelines.org.uk/index.aspx?Publication=RB

Guidance from The Royal College of Pathologists and the Institute of Biomedical Science 'The retention and storage of pathological records and archives' 4th edition (2009)
http://www.rcpath.org/publications-media/publications/publications.htm#general

Human Tissue Authority Codes of Practice
http://www.hta.gov.uk/legislationpoliciesandcodesofpractice/codesofpractice.cfm

International Standards for Cellular Therapy Product Collection, Processing and Administration 5th edition (2012) FACT-JACIE
http://www.factweb.org/forms/store/ProductFormPublic/search?action=1&Product_productNumber=621

Renal Association Guidelines: Assessment of the Potential Kidney Transplant Recipient 5th edition (2011) http://www.renal.org/Clinical/GuidelinesSection/AssessmentforRenalTransplantation.aspx

Standards for cord blood collection, processing, testing, banking, selection and release 5th edition (2013) NetCord-FACT
http://www.factweb.org/forms/store/ProductFormPublic/search?action=1&Product_productNumber=627

Standards for Histocompatibility Testing Version 6.1 (2013) EFI
http://www.efiweb.eu/index.php?id=102

All links verified 06/01/2015.

AiT	Antibody incompatible Transplantation
AIN	Autoimmune Neutropenia
AITP	Autoimmune Thrombocytopenia
ALI	Acute Lung Injury
ANI	Autoimmune Neutropenia of Infancy
ARDS	Acute Respiratory Distress Syndrome
BBMR	British Bone Marrow Registry
BSS	Bernard Soulier Syndrome
CPA	Clinical Pathology Accreditation
CIT	Cold Ischemia Time
DDITP	Drug Dependent Immune Thrombocytopenia
DH	Department of Health
DSA	Donor Specific Antibodies
EFI	European Federation of Immunogenetics
FCXM	Flow Cytometric Crossmatch
FFP	Fresh Frozen Plasma
GCLT	Granulocyte Chemiluminescence Test
GI	Granulocyte Immunology
GIFT	Granulocyte Immunofluorescence Test
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GT	Glanzmann's Thrombasthenia
H&I	Histocompatibility & Immunogenetics
HIT	Heparin Induced Thrombocytopenia
HLA	Human Leukocyte Antigen
HNA	Human Neutrophil Antigen
HPA	Human Platelet Antigen
HTA	Human Tissue Authority
MHRA	Medicines and Healthcare products Regulatory Agency
MAIGA	Monoclonal Antibody Immobilisation of Granulocyte Antigen
MAIPA	Monoclonal Antibody Immobilisation of Platelet Antigen
NAIN	Neonatal Alloimmune Neutropenia
NAIT	Neonatal Alloimmune Thrombocytopenia
NHSBT	National Health Service Blood and Transplant
NHFTR	Non-Haemolytic Febrile Transfusion Reaction
ODT	Organ Donation and Transplantation
PAIg	Platelet Associated Immunoglobulin
PI	Platelet Immunology
PIFT	Platelet Immunofluorescence Test
PTP	Post Transfusion Purpura
SHOT	Serious Hazards of Transfusion
SOP	Standard Operating Procedure
STR	Short Tandem Repeat
SSOP	Sequence Specific Oligonucleotide Probing
SSP	Sequence Specific Priming
TA-GVHD	Transfusion Associated Graft Versus Host Disease
TRALI	Transfusion Related Acute Lung Injury
UK NEQAS	United Kingdom National External Quality Assurance Scheme